Simple Staining Lab

Introduction and Background:

Microbes are too small to see with the naked eye and are often colorless. As a result, they must be first stained and then studied using a microscopy in order to see them and categorize them appropriately. The stain is substance that will be able to bind to the cell and display its particular color. There are many different stains with different colors that have different affinities for different organisms. Stains can even be used to differentiate parts of the cell itself.

In microbiology there are several types of stains that are used. The first is a **simple stain**. The simple stain will use just use one reagent and provides contrast between the background and the microbe itself. The microbe, which was originally colorless will adhere to the stain and become colored. The background should remain unstained due to the washing the slide with distilled water that will be explained later.

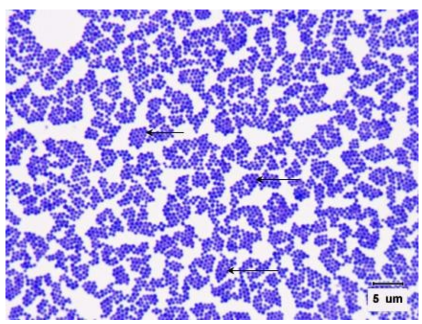


Image 1: Staphylococcus species with crystal violet only (1,000X)

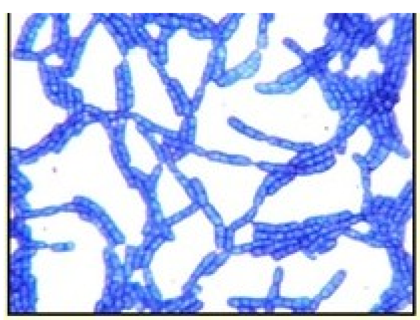


Image 2: Bacillus species with methylene blue (1,000X)

<u>Pre-lab question:</u>

1. *What kind of characteristics can be defined using a simple stain?*

Another type of stain is the **negative stain**. This will use a single stain to provide contrast between the background and the microbe. In other words, the background should appear “black” or “stained”, but the microbe will not take up any stain and appear clear.

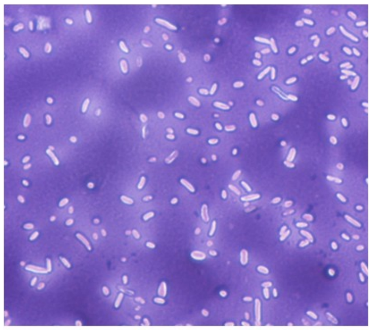


Image 3: Bacillus species with negative stain (1,000X)

There is also more complicated staining procedure that can be used to differentiate structures of a microbe. These types of stains are said to be **differential stain.** With a differential stain you will typically uses two or more reagents. There will be a primary stain, a wash, and then a counter stain will be applied to the microbes.

The stains that are applied are typically chemically different. You can have two types of stains: **basic stains**, which have a positive charge and **acidic stains**, which have a negative charge.

Cells have various charges and these stains will be able to bind onto their structures due to their charges. Basic stains (cataionic) have an affinity for negatively charged structures of the microbe. The types of dyes that are basic include methylene blue, crystal violet, and carbol fuchsin.

Acidic stains (anionic) have an affinity for positive charges structures of the microbe. The types of dyes that are acidic include India ink and nigrosin. An example of this phenomenon occurs with a bacteria’s cell wall. The cell wall is negatively charged, so a positive dye will be attracted to it. If a negative dye were added first, the dye would be repulsed by the cell wall due to its negative nature and will not

directly stain the cell.

Pre-lab question:

2. *What charge does a cationic stain have?*

3. *What charge does an anionic stain have?*

Materials:

- Wax Pencils (6 per Table)

- Distilled Water Bottles (2 per table)

- Disinfectant Bottles (2 per table)

- Test Tube Racks (2 per Table)

- Metal Inoculating Loops (6 per Table)

- Metal Inoculating Needles per table (6 per Table)

- Bunsen Burners and Hoses (2 of each per table)

- Boxes of Microscope Slides (1 per table)

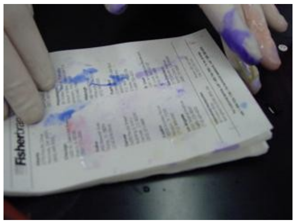
- "Waste" 500ml Beakers (1 per table)

- Simple Staining Kit (2 per table)
  - Each kit should have the following
    - Methelyne Blue

- Optical Lens Paper (3 per table)

- Metal Staining Trays with Racks (6 per table or as many as available)

- Blotting Paper if available (6 per table) or Large Brown Paper Towels Rolls (1 per table)



- 2 test tubes with Organism #1
- 2 test tubes with Organism #2
- 2 test tubes with Organism #3

Procedure:

Step 1 – Preparation of Microbial Specimen on a Glass Slide

The microbes that you have been provided to you by your instructor need to be placed on a glass slide and then a dye has to be added to them. In order for the microbes to NOT be washed away, it is going to be your responsibility to “fix” them on the slide. This “fixing” is accomplished by preparing a **microbial smear**. A smear by definition is a loopful of the microbe medium that is to be spread using your metal loop (smeared) onto a microscope slide. It is important to note that you do not want to put too many microbes on the slide. If you do put too many microbes on the slide, you not be able to identify a single cell. Instead, you will be looking at a pancake of microbes, one on top of the other and will not be able to differentiate any characteristics of the microbe while using the microscope.

<u>Pre-lab question:</u>

4. *Why do you not want to put too many microbes on the slide?*
5. *What will the simple staining procedure help you identify underneath the microscope?*

In this class, you will be using heat fixation on the microbial smear. This heat fixation will be able to do three specific tasks. First, the microbes will be killed. Second, the organisms will not move from the slide once you add the dye. Finally, the organisms will be able to accept the dyes easier.

1. Obtain a glass slide.

2. If you are using a **broth culture**:

a. Gently tap your culture broth tube to disperse the microbes throughout the entire broth.

b. Sterilize your inoculating loop using a Bunsen burner. Make sure that your Bunsen burner is secured. It should never be left unattended at any time. Let the inoculating loop cool for 20-30 seconds.

c. Take your sterile inoculating loop and remove the microbes aseptically by putting the loop in the broth.

d. Take the loop and rub it on the glass slide. Make sure to rub the loop about a nickel in diameter.

e. Sterilize the loop again to ensure that you have killed any microbes.

f. The smear should now be set on the side so that it can air dry completely. Do not use heat to dry your smear.

3. Using an **agar plate or slants**:

a. Place a **small** drop of water in the center of the slide.

b. Sterilize your inoculating loop using a Bunsen burner. Make sure that your Bunsen burner is secured. It should never be left unattended at any time. Let the inoculating loop cool for 20-30 seconds.

c. Take your sterile inoculating loop and remove the microbes aseptically by taking the loop and touching the microbial colonies that are present. ***DO NOT DIG INTO THE AGAR.*** All you have to do is just gently touch the microbes with your loop.

d. Take the loop and rub it with the drop of water on the glass slide. Make sure to rub the loop about a nickel in diameter.

e. Sterilize the loop again to ensure that you have killed any microbes.

f. The smear should now be set on the side so that it can air

dry completely. Do not use heat to dry your smear.

4. Heat fix the slide. By using the Bunsen burner method. Hold the slide with a wooden clothes pin, and pass 10- 12 times through the flame. Let the slide cool.

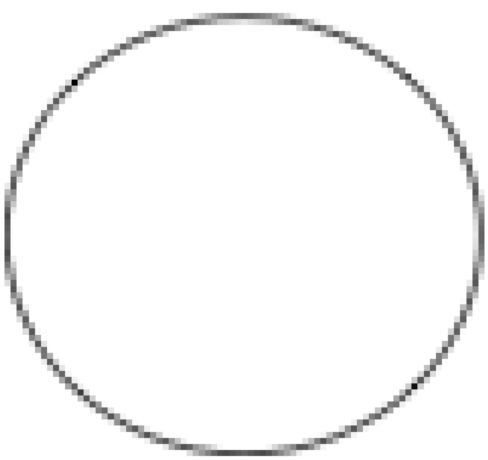
5. Optional step: Once the slide has cooled please use a wax pencil to outline the area of the smear.

6. We will be using methylene blue as the staining reagent in this laboratory. Flood your smear with methylene blue and allow it to stand for 1 minute

7. Briefly wash off the methylene blue stain with distilled water (remember this is not a pressure wash! Be gentle).

8. Place your slide within your book of bibulous paper and GENTLY blot the water drops off of the slide.
9. Use your microscope to identify the microorganisms using High Power and Oil Immersion Power. Draw your results below and write your observations for each microorganism.

RESULTS:



400X *Organism #1:*______________

400X *Organism*

*#2:*_______________

400X *Organism*

*#3:*_______________

1000X *Organism*

*#1:*_______________

1000X *Organism*

*#2:*________________

1000X *Organism*

*#3:*________________

Observations

Organism #1:_______________

Organism #2:__________________

Organism #3:__________________

Post-Lab Questions:

1. Why did you have to heat fix the microbes to the slide?

2. List and explain the different types of flagella that microorganisms can have.

3. What are the shapes that microorganisms can come in? Use their scientific term to describe their shape.

4. Can you see organelles in a yeast cell? If so, list the organelles that you saw and describe their function.

5. Did you physically see the plasma membrane, yes or no? If yes, describe it. If no, what did you see instead?